Supplemental Information

Extracellular vesicle-contained eNAMPT delays aging and extends lifespan in mice

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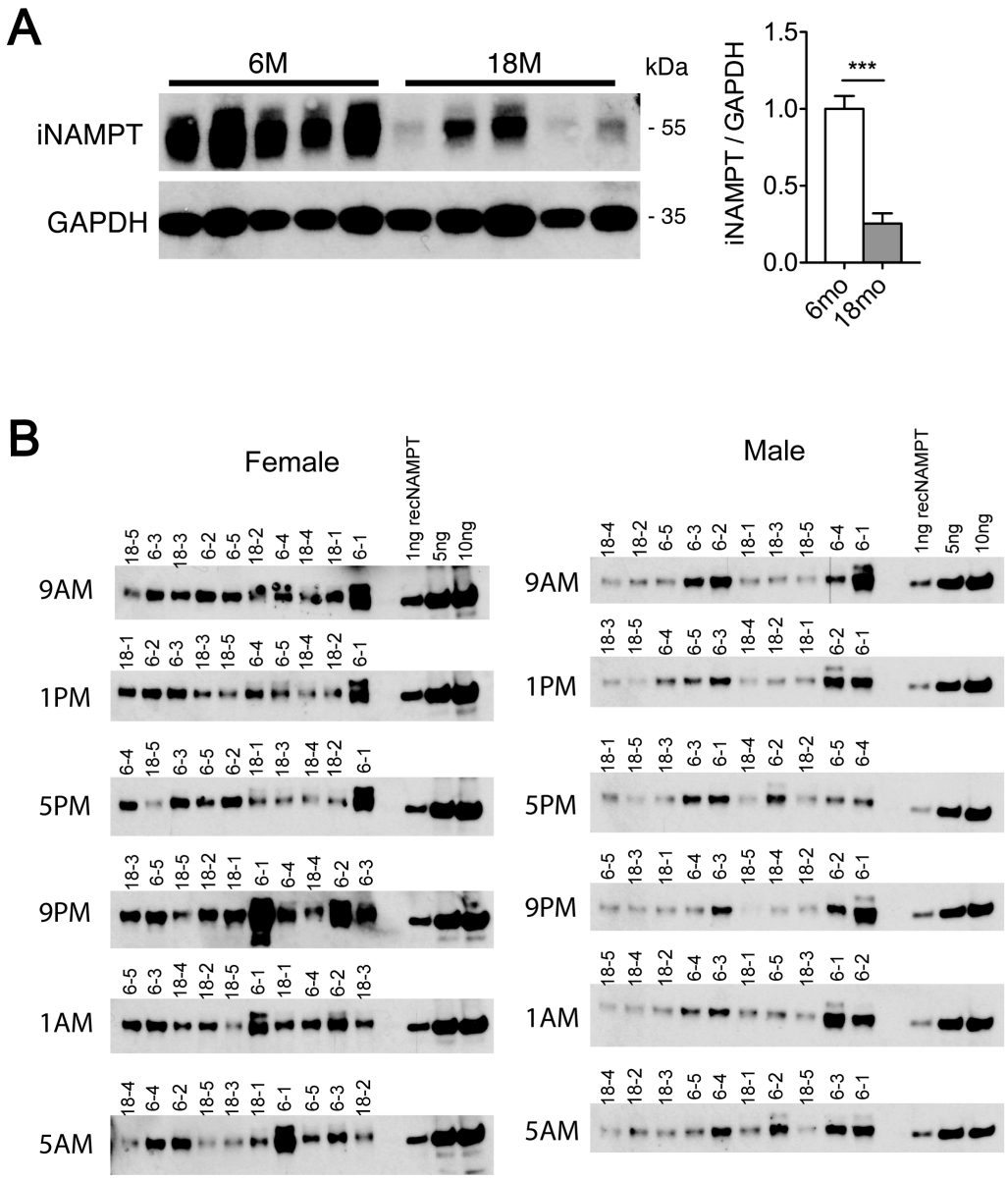

Figure S1; *Related to Figure 1*: Adipocyte and plasma NAMPT levels significantly decline with age.

(A) NAMPT levels in primary adipocytes isolated from 6 and 18 month-old male mice (n=5).

(B) Western blots for plasma eNAMPT levels over the course of 24 hrs in male and female mice at 6 and 18 months of age (n=5 per time point per age). 6-1~5 and 18-1~5 are individual plasma samples at 6 and 18 months of age, respectively. The order of samples in each blot was intentionally randomized to avoid biases in signal quantitation.

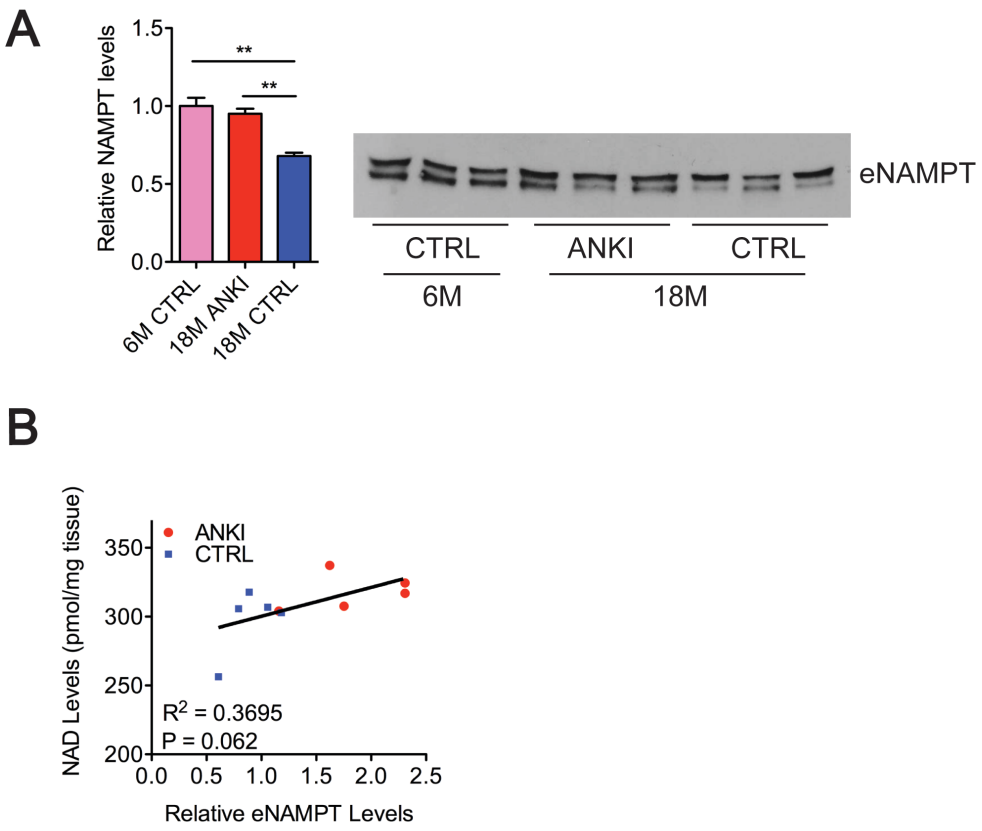

Figure S2; *Related to Figure 2*: Adipose tissue-specific overexpression of NAMPT increases plasma eNAMPT levels and tissue NAD$^+$ biosynthesis.
(A) Plasma eNAMPT levels of 6 month-old control, 18 month-old ANKI, and 18 month-old control mice (n=3). Plasma eNAMPT shows doublet bands (right panel), both of which were quantitated (left panel).
(B) Relationship of plasma eNAMPT levels and hypothalamic NAD$^+$ levels in control and ANKI female mice at 20 months of age (n=5 per group).

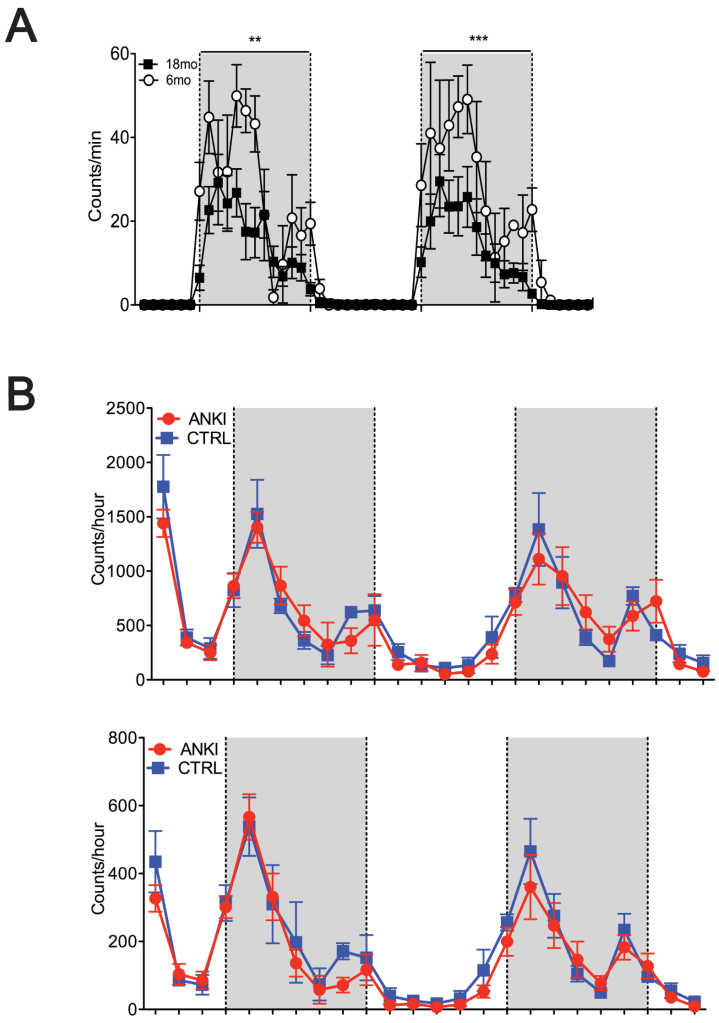

Figure S3; *Related to Figure 3*: ANKI male mice show no difference in total ambulatory and rearing activities.

(A) Wheel-running activity of 6 and 18 month-old wild-type mice (n=3-9). Differences were assessed by Wilcoxon matched-pairs singled-ranked test.

(B) Ambulatory (top) and rearing (bottom) activities of control (CTRL) and ANKI male mice at 18 months of age (n=4-7).

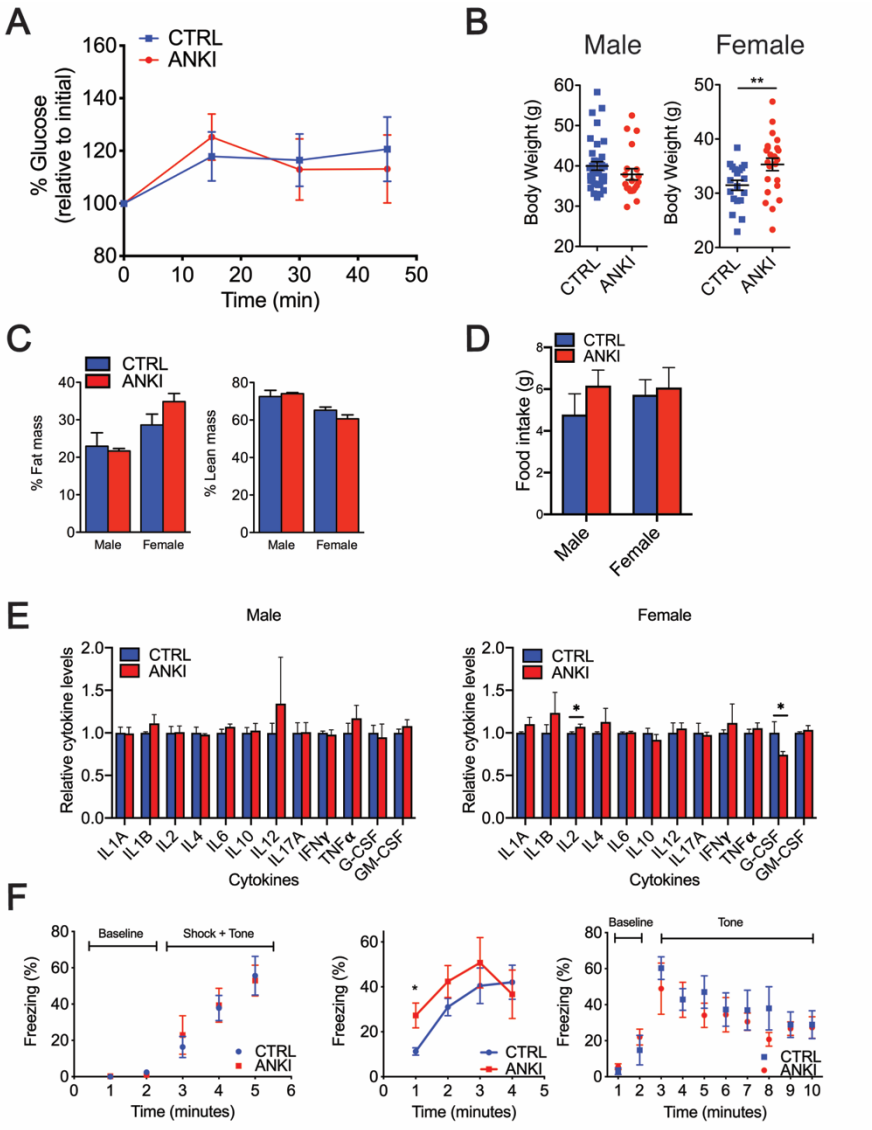

Figure S4; *Related to Figure 4*: insulin tolerance, body weight, body composition, food intake proinflammatory cytokine levels, and contextual fear conditioning between aged control and ANKI mice.

(A) Relative blood glucose levels of control (CTRL) and ANKI male mice at 17-20 months of age during insulin tolerance tests (n=8 per group). Glucose levels at each time point are normalized to that at 0 min time point.

(B) Body weights of control and ANKI female and male mice at the age of 18-20 months of age (male, n=20-36; female, n=19-23).

(C) Body compositions of control CTRL and ANKI male and female mice at 17-20 months of age.

(D) Daily food intakes of control and ANKI male and female mice at 23 months of age (n=6-9).

(E) Relative plasma cytokine levels of control and ANKI male and female mice at 23 months of age (n=3).

(F) Contextual fear conditioning test of control and ANKI female mice at 20 months of age. Left panel: Percent freezing time of control and ANKI mice at baseline and during shock-tone training; Middle panel: Contextual fear response on day2; Right panel: Baseline and auditory cue response on day3 (n=5).

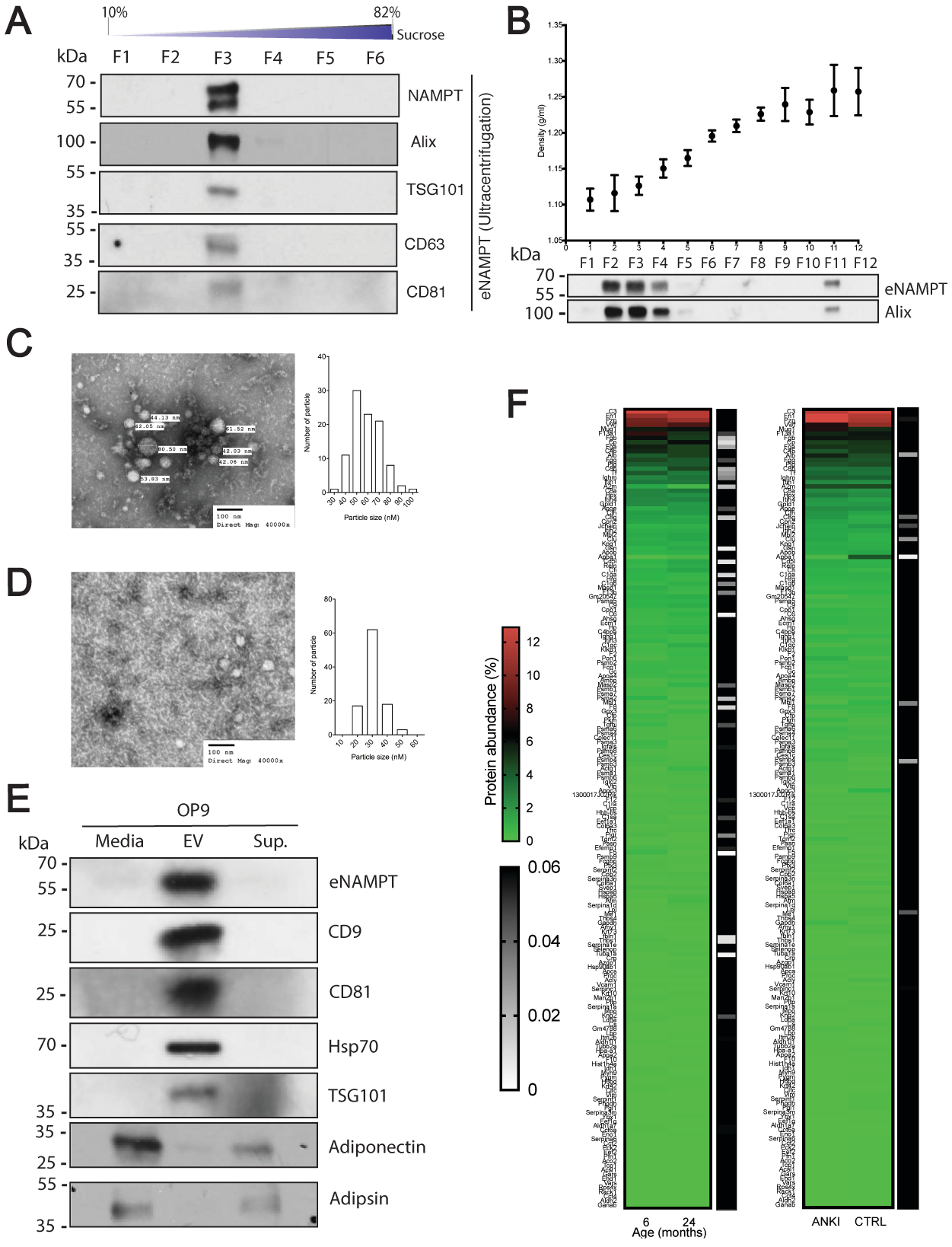
A
10%
82%
Sucrose
kDa
F1
F2
F3
F4
F5
F6
70 -
55 -
NAMPT
100 -
Alix
55 -
TSG101
35 -
55 -
CD63
35 -
25 -
CD81
eNAMPT (Ultracentrifugation)
B
Density (g/ml)
kDa
F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12
70 -
55 -
eNAMPT
100 -
Alix
C
Number of particle
Particle size (nM)
D
Number of particle
Particle size (nM)
E
OP9
kDa
Media
EV
Sup.
70 -
55 -
eNAMPT
25 -
CD9
25 -
CD81
70 -
Hsp70
TSG101
35 -
35 -
Adiponectin
25 -
55 -
Adipsin
35 -
F
Protein abundance (%)
12
10
8
6
4
2
0
0.06
0.04
0.02
0
6
24
Age (months)
ANKI
CTRL

Figure S5; *Related to Figure 6*: Characteristics of EVs isolated from mouse and human plasma.
(A) eNAMPT and EV marker proteins in six fractions isolated by flotation of ultracentrifugation-purified EVs into a sucrose-density gradient.
(B) Densities of 12 fractions and the comparison of eNAMPT and an EV marker Alix in each fraction isolated from a sucrose-density gradient separation of EVs purified by the Total Exosome Isolation (TEI) kit. A very minor fractions of eNAMPT and Alix, which were cofractionated in Fraction #11, are most likely due to a contamination of protein aggregates.
(C, D) Electron microscopic images and particle size distributions of EVs isolated from mouse (C) and human plasma (D) (n=100 for each analysis).
(E) Comparison of eNAMPT, EV marker proteins (CD9, C81, Hsp70, and TSG101), and non-EV proteins (adiponectin and adipsin) in the conditioned media of OP9 adipocytes. The EV fraction and supernatant were separated by ultracentrifugation. 40 μg of protein from each fraction was loaded.
(F) The heatmap of the proteins detected in proteomic analyses of the EVs isolated from 6 and 24 month-old female mice (left panel) and 24 month-old control (CTRL) and ANKI female mice (right panel).

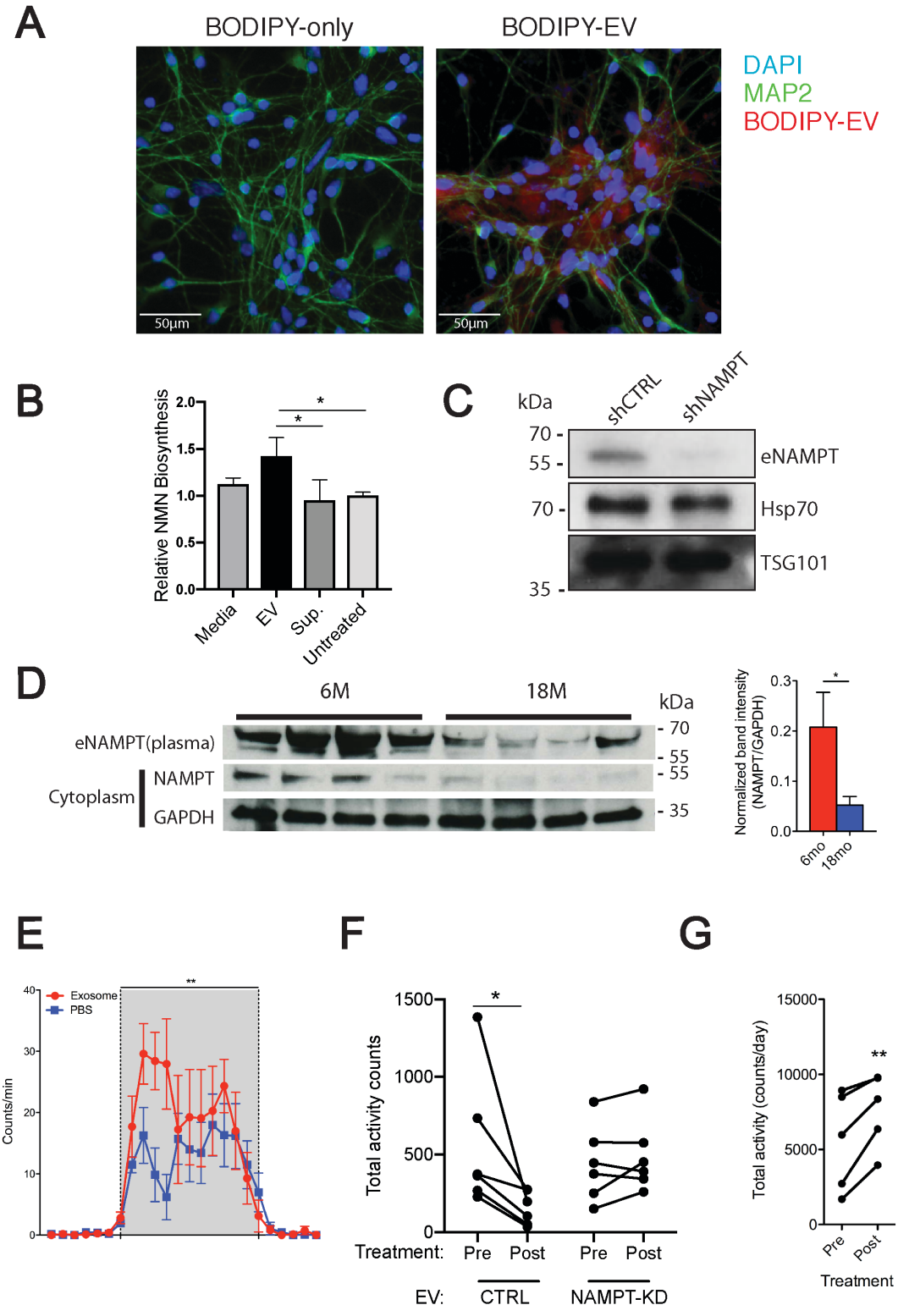
A
BODIPY-only
BODIPY-EV
DAPI
MAP2
BODIPY-EV
50μm
50μm
B
Relative NMN Biosynthesis
2.0
1.5
1.0
0.5
0.0
*
*
Media
EV
Sup.
Untreated
C
kDa
shCTRL
shNAMPT
70 -
55 -
eNAMPT
70 -
Hsp70
TSG101
35 -
D
6M
18M
kDa
eNAMPT(plasma)
- 70
- 55
Cytoplasm
NAMPT
- 55
GAPDH
- 35
Normalized band intensity (NAMPT/GAPDH)
0.3
0.2
0.1
0.0
*
6mo
18mo
E
Exosome
PBS
**
Counts/min
40
30
20
10
0
F
Total activity counts
1500
1000
500
0
*
Treatment:
Pre
Post
Pre
Post
EV:
CTRL
NAMPT-KD
G
Total activity (counts/day)
15000
10000
5000
0
**
Pre
Post
Treatment

Figure S6; *Related to Figure 7*: EV-contained eNAMPT is internalized to target cells and ameliorates age-associated decline in wheel-running activity.
(A) Fluorescent images of primary hypothalamic neurons following the incubation with BODIPY-labeled EVs from OP9 adipocytes.
(B) Relative NAMPT enzymatic activity in primary hypothalamic neurons after incubating with each fractions (Media, EV, and supernatant) isolated from the conditioned media of OP9 adipocytes. Each NMN biosynthesis level measured by mass spectrometry with D-4-NAM was normalized to that in untreated cells.
(C) eNAMPT levels in EVs isolated from control and *Nampt*-knockdown (NAMPT-KD) OP9 adipocytes. Protein concentrations of EVs purified from both conditioned media were very similar, suggesting that there was no difference in the amounts of EVs released from both cell lines.
(D) Levels of cytoplasmic NAMPT in primary hypothalamic neurons after incubated with plasma from 6 and 18 month-old mice.
(E) Total wheel-running activity counts throughout 24 hrs of 20 month-old female mice before and after four consecutive daily injections of EVs isolated from 4-6-month old mice (n=5).
(F) Total wheel-running activity counts of 25 month-old female mice during the light time before and after 4 consecutive daily injections of EVs purified from control (CTRL) and NAMPT-KD OP9 adipocytes (n=5-6).
(G) Total wheel-running activity counts of 20 month-old male mice during dark and light times before and after 4 consecutive daily injections of EVs purified from 4-6-month old mice (n=5).